

Morphometrics analysis on the hind wing of Tetrigidae (Orthoptera) and its application in taxonomy

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Abstract: The morphological characteristics of the hind wing venation of tetrigids were analyzed on the basis of morphometric measurement, with 20 species and 18 different variables selected for this study. Results show that three principal components have a higher load at the length between the starting point of the costal vein and the tip of the fourth anal vein, between the tip of the costal vein and the tip of the third anal vein, and between the tip of the costal vein and the tip of the eleventh anal vein. There exists a contrast between wing length and width.

Key words: morphometric measurement; insect morphology; evolutionary development

蚱类昆虫后翅的形态测量学研究（直翅目）

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摘要: 选取 20 种蚱类昆虫作为研究材料, 通过标点法选取 18 个翅脉相关变量对蚱类昆虫后翅翅脉的形态特征进行形态测量学分析。结果表明: 得出的 3 个主成分在前缘脉 C 起点到臀脉 4A 点长、前缘脉 C 顶点到臀脉 3A 点长、前缘脉 C 顶点到臀脉 11A 点长、翅长与翅宽之比上有较高的载荷。

关键词: 形态测量; 昆虫形态; 进化

Introduction

In classical taxonomy, the wing nervures of insects are commonly used as important taxonomic indicators for identification at the level of families and genera (Pan *et al.* 2008). Although some differences exist in the wing nervure of different orders, it is difficult to make an accurate judgment. Therefore, geometric morphometrics has been widely used for insect taxonomy (Rohlf 1993; Zhao *et al.* 2003; Bai & Yang 2007; Yan & Hua 2010). The insect exoskeleton and wings are easy to measure, and several software programs are used for their analysis. However, some researchers use geometric morphometrics to study the taxonomy and phylogeny of insect (Rohlf 1993; Trueman 1996; Güler *et al.* 2006; Villemant *et al.* 2007;

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Bubliy *et al.* 2008; Pan *et al.* 2008; Feng *et al.* 2010; Cheng *et al.* 2010). For example, Güler *et al.* (2006) used it to study *Anthidium* species (Hymenoptera: Megachilidae); Villemant *et al.* (2007) used it to study *Eubazus* species (Hymenoptera: Braconidae); Bubliy *et al.* (2008) used geometric morphometrics to classify the *virilis* group of *Drosophila*; and Prieto *et al.* (2009) used it to study *Cupido minimus* and *C. carswelli* (Lepidoptera: Lycaenidae) and to discriminate whether these two species are identical.

Tetrigids belong to Tetrigoidea, Orthoptera and most species have two pairs of wings, similar to most other winged insects. Hind wing nervure is an important characteristic in interspecies taxonomy. Because different species have different morphological characteristics, and each species has a unique venation, the morphometrics of hind wing nervures can be used for taxonomy.

In this study, we apply morphometrics and principal component analysis to study hind wing venation in Tetrigidae, to explore the interspecific differences, and to validate the relationship of hind wing venation among these twenty species. Furthermore, principal component analysis was used to determine the phylogenetic relationships among the 20 species of Tetrigidae.

Material and methods

Specimen Selection

Specimens were selected in two ways. Some specimens were obtained from the Insect Museum of Hechi University, Yizhou, China. Others were collected from Shiwanshan Nature Reserve, Guangxi Autonomous Region, by Weian DENG and Rongjiao ZHANG. Twenty species in 11 genera are represented in this analyses. Ten of each species were selected and all specimens were mature females (compared with males, there is little change in hind wing venation in mature females). The twenty species are listed in Table 1. All specimens are deposited in the Insect Collection of Hechi University.

Table 1. Twenty species information

Family	Genus	Species
Metrodoridae	<i>Tetrix</i> Latreille, 1802	<i>Tetrix xiangzhouensis</i> (Deng, Zheng & Wei, 2008)
	<i>Systolederus</i> Bolivar, 1887	<i>Systolederus longipennis</i> (Zheng & Jiang, 2004)
	<i>Bolivaritettix</i> Gunther, 1939	<i>Bolivaritettix circinihumerus</i> (Zheng, 2003)
		<i>Bolivaritettix torulosinota</i> (Zheng, 2005)
		<i>Bolivaritettix sikkimensis</i> (Bolivar, 1909)
Tetrigidae		<i>Bolivaritettix guibeiensis</i> Deng, Zheng & Wei, 2007
	<i>Teredorus</i> Hancock, 1906	<i>Teredorus guangxiensis</i> (Zheng & Deng, 2004)
	<i>Coptotettix</i> Bolivar, 1887	<i>Coptotettix longjiangensis</i> (Zheng & Wei, 2000)
	<i>Hedotettix</i> Bolivar, 1887	<i>Hedotettix latifemuroides</i> (Zheng & Jiang, 2004)
	<i>Tetrix</i> Latreille, 1802	<i>Tetrix interrupta</i> (Zheng & Xu, 2010)
	<i>Euparatettix</i> Hancock, 1904	<i>Euparatettix variabilis</i> (Bolivar, 1887)
		<i>Euparatettix bimaculatus</i> (Zheng, 1993)
		<i>Euparatettix jiuwanshanensis</i> (Zheng & Deng, 2004)

Continued Table 1.

Family	Genus	Species
Scelimenidae	<i>Eucriotettix</i> Hebard, 1929	<i>Eucriotettix strictivertex</i> (Deng & Zheng, 2012)
		<i>Eucriotettix grandis</i> (Hancock, 1912)
	<i>Criotettix</i> Bolivar, 1887	<i>Criotettix bispinosus</i> (Dalman, 1818)
		<i>Criotettix japonicus</i> (De Haan, 1842)
	<i>Zhengitettix</i> Liang, 1994	<i>Zhengitettix curvispinus</i> (Liang, Jiang & Liu, 2007)
		<i>Zhengitettix nigrofemurus</i> (Deng, Zheng & Wei, 2010)

Methods

The dried specimens were softened by inserting them in a foam board and placing them in 100°C boiling water for 5 min. Subsequently, they were removed from water and their surface moisture was dried with absorbent paper. The right hind wing was treated first. Initially, the wing was gently removed by surgical tweezers, placed in 75% ethanol, and soaked for 3 min for surface infiltration and flexibility. Next, the wing was transferred to warm water at 60°C for 1 min to allow complete extension of the wing. After removing from water, the wing was adhered to a piece of paper (15 cm) that was wider than the wing and the excess water was eliminated by drainage. If the paper and wing were not completely dried, they were pressed together with prepared paper and then placed under books or other flat heavy objects until they were fixed and dried. Finally, the layer of paper was opened with a brush, dipped in 95% ethanol, and the paper was wetted on the opposite side of the wing. When the ethanol naturally penetrated the entire piece of paper on both sides, the wing fell off and was ready for analysis.

Data Acquisition

The picture of the hind wings was acquired using a CANON CB-2LZE digital camera. The coordinates of 18 landmarks were recorded on the digitized wing picture using TPSDIG2 (Rohlf 2010) software (Figure 1). All measurements were made using an electronic digital display (flash) caliper (0–150 mm/6"; Guilin Guanglu Measuring Instrument Co.). To decrease digitization noise, each species was measured 10 times and each wing was measured six times using the same image, and the results were averaged. Wing venation

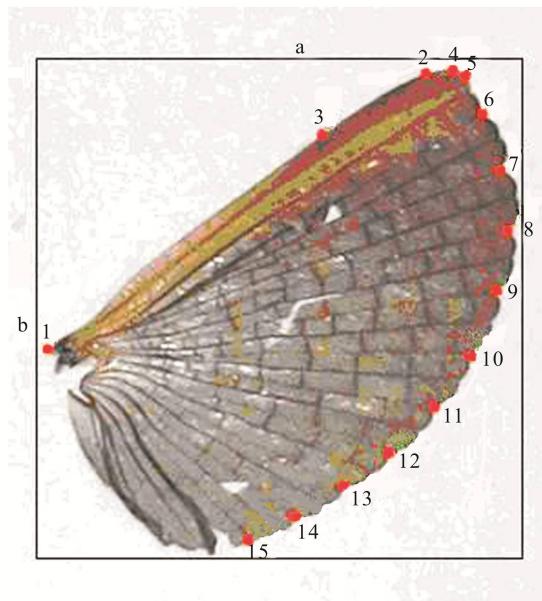


Figure 1. The morphometric landmarks on the hind wings of tettigids.

Note: the numbers and letters in the figure indicate the selected landmark points (landmarks).

nomenclature follows Deng *et al.* (2007). To eliminate the possible impact of different units, the data were standardized prior to analysis by SPSS19.0 (Xie *et al.* 2012) software (Table 2).

Table 2. Distance of landmarks

Variable quantities	Measured distance	Definition
V1	1–2	length of costal vein
V2	1–3	length of subcostal vein
V3	1–4	length of cubital vein
V4	1–5	length of first anal vein
V5	1–6	length between starting point of costal vein and tip of second anal vein
V6	1–8	the length between the starting point of the costal vein and the tip of the fourth anal vein
V7	1–10	the length between the starting point of the costal vein and the tip of the sixth anal vein
V8	1–12	the length between the starting point of the costal vein and the tip of the eighth anal vein
V9	1–14	the length between the starting point of the costal vein and the tip of the tenth anal vein
V10	2–4	the length between the tip of the costal vein and the tip of the cubital vein
V11	2–5	the length between the tip of the costal vein and the tip of the first anal vein
V12	2–7	the length between the tip of the costal vein and the tip of the third anal vein
V13	2–9	the length between the tip of the costal vein and the tip of the fifth anal vein
V14	2–11	the length between the tip of the costal vein and the tip of the seventh anal vein
V15	2–13	the length between the tip of the costal vein and the tip of the ninth anal vein
V16	2–15	the length between the tip of the costal vein and the tip of the eleventh anal vein
V17	a	the length of wings
V18	a/b	a contrast between wing length and width

Results and analyses

KMO and Bartlett's test: $\text{Sig} = 0.000$ by Bartlett's test of sphericity indicated that the data reject the null hypothesis and that the morphological characteristics were significantly different between each group ($P < 0.001$); KMO = 0.655, which was close to 0.7 (Table 3), met the prerequisite factor of analysis. The results demonstrated a strong correlation between the variable quantities and the data were suitable for factor analyses.

Table 3. KMO and Bartlett's Test

Kaiser–Meyer–Olkin Measure of Sampling Adequacy	0.655
	Approximate Chi-Square
Bartlett's Test of Sphericity	Df
	Sig.

Factor analyses

We analyzed 18 variable quantities of 20 species of tetrigids using SPSS 19.0 software and determined the main components of total variance (Table 4). The value of the first three

components, which are considered the main factors, was 87.360%. Moreover, the factor loading matrix was also determined from a principal component analysis by using this factor analysis.

Table 4. Total variance explained by the first three principal components for principal component analysis

Component	Initial Eigen values			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	13.360	74.223	74.223	13.360	74.223	74.223
2	1.245	6.918	81.141	1.245	6.918	81.141
3	1.119	6.219	87.360	1.119	6.219	87.360

PCA results

We used the SPSS 19.0 software to obtain the feature vector matrix (Table 5) from the factor loading matrix through principal component analysis.

Table 5. The Feature Vector Matrix^a

Variable quantities	T1	T2	T3
V1	0.2884	-0.0653	0.0426
V2	0.2821	-0.0138	-0.0258
V3	0.2887	-0.0723	0.0344
V4	0.2902	-0.0685	0.0395
V5	0.2902	-0.0724	0.0338
V6	0.2918	-0.0735	0.0089
V7	0.2855	-0.0718	-0.0336
V8	0.2558	0.0013	-0.115
V9	0.2413	0.0335	-0.1146
V10	0.2296	0.0328	0.052
V11	0.1986	-0.0464	0.0305
V12	0.2228	0.1723	-0.0258
V13	0.2566	0.1387	-0.0376
V14	0.2596	0.1254	-0.0009
V15	0.2733	0.0758	0.0155
V16	0.2912	0.0029	0.0068
V17	0.2865	-0.0497	0.0056
V18	0.0706	0.0832	0.2506

Based on the feature vector matrix, we observed that there was a high load at V6 and V16 in the first principal component. V6 measured the distance between 1–8 points, referring to the length between the starting point of the costal vein and the tip of the fourth anal vein, whereas V16 measured the distance between 2–15 points, referring to the length between the tip of the costal vein and the tip of the eleventh anal vein. It is known that the larger the absolute values of the load factor, the greater the contribution. This indicates that the first principal component was dominated by two factors: the length between the starting point of the costal vein and the tip of the fourth anal vein, and the length between the tip of the costal vein and the tip of the eleventh anal vein. In addition, there was a high load at V12 in the second principal

component. V12 measured the distance between 2–7 points, referring to the length between the tip of the costal vein and the tip of the third anal vein. This indicates that the second principal component was dominated by the length between the tip of the costal vein and the tip of the third anal vein. Similarly, there was a high load at V18 in the third principal component. V18 measured the contrast between wing length and width, indicating that the third principal component was dominated by the contrast between wing length and width.

The scores of the three main components were obtained through the feature vector matrix (Table 6), and the scatter diagram based on the score of the three main components could obviously distinguish 20 species of tetrigids (Figure 2).

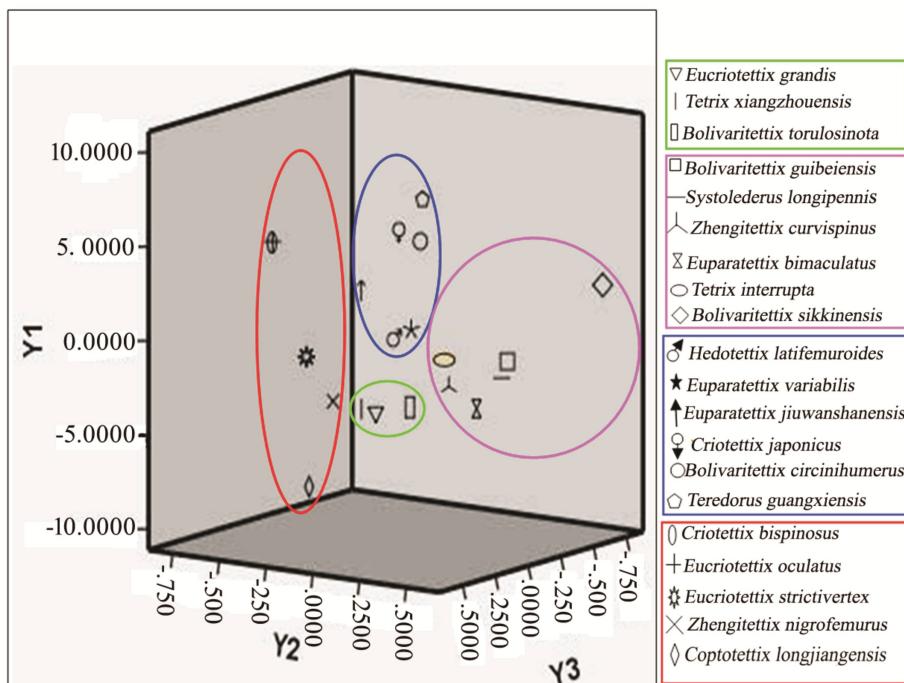


Figure 2. The scatter diagram of the score of the three main components.

Table 6. The component score of 20 species of hind wing venation of Tetrigidae

Species	Y1	Y2	Y3
<i>Bolivaritettix torulosinota</i>	-3.4704	0.0721	0.0384
<i>Systolederus longipennis</i>	-2.5078	0.2677	-0.3843
<i>Eucrotettix grandis</i>	-2.3568	0.2656	0.5754
<i>Tetrix interrupta</i>	-0.484	0.3054	0.1088
<i>Euparatettix bimaculatus</i>	-3.2231	0.3858	-0.022
<i>Criotettix bispinosus</i>	4.0794	-0.6933	-0.0013
<i>Teredorus guangxiensis</i>	7.6444	0.1372	0.0354
<i>Bolivaritettix guibeensis</i>	-1.0353	0.4241	-0.2034
<i>Zhengitettix nigrofemurus</i>	-3.9026	-0.3731	-0.0104
<i>Euparatettix jiuwanshanensis</i>	1.6841	-0.336	-0.1723
<i>Coptotettix longjiangensis</i>	-9.0151	-0.5918	-0.1398

Continued Table 6.

Species	Y1	Y2	Y3
<i>Hedotettix latifemuroides</i>	0.7206	0.1507	0.2786
<i>Criotettix japonicus</i>	4.6205	-0.2743	-0.372
<i>Euparatettix variabilis</i>	2.1133	0.4092	0.5103
<i>Zhengitettix curvispinus</i>	-3.553	-0.056	-0.4419
<i>Bolivaritettix sikkimensis</i>	2.1784	0.5725	-0.7194
<i>Eucriotettix strictivertex</i>	-1.1884	-0.3906	0.1684
<i>Tetrix xiangzhouensis</i>	-2.3651	0.1741	0.5536
<i>Eucriotettix oculatus</i>	4.9703	-0.5005	0.2686
<i>Bolivaritettix circinohumerus</i>	5.0906	0.0511	-0.0708

Cluster Analyses

The 20 species of tetrigids were cluster analyzed using principle component analyses described above (Wu *et al.* 2007). The results are shown in Figure 3.

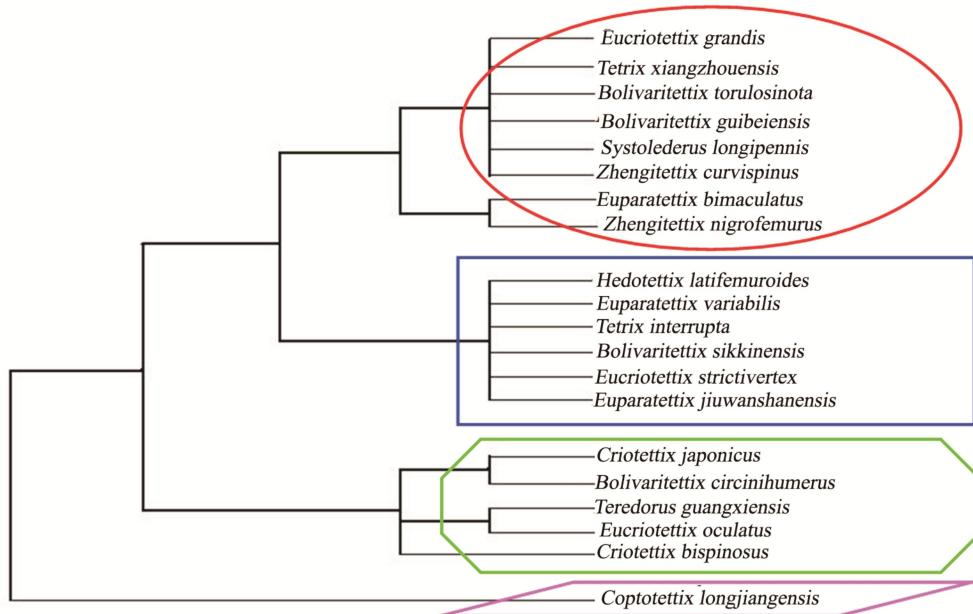


Figure 3. The results of cluster analyses of 20 species of Tetrigidae.

The 20 species clustered into four groups. The first group included eight species: *Euparatettix bimaculatus* and *Zhengitettix nigrofemurus* were clustered into a small group; they subsequently cluster into a bigger group with *Eucriotettix grandis*, *Tetrix xiangzhouensis*, *Bolivaritettix torulosinota*, *B. guibeensis*, *Systolederus longipennis*, and *Z. curvispinus*. The second group included six species: *Hedotettix latifemuroides*, *E. variabilis*, *T. interrupta*, *B. sikkimensis*, *Eucriotettix strictivertex*, and *E. jiuwanshanensis*. The third group included five species: *Criotettix japonicus* and *B. circinohumerus* were clustered into a small group and

Teredorus guangxiensis and *Eucriotettix oculatus* were clustered into another small group; these two small groups were subsequently clustered into a big group along with *Criotettix bispinosus*. *Coptotettix longjiangensis* formed the fourth group. It validated that each group had highly similar hind wing venation among the members of its own group. Further, the first group had the highest similarity with the second group, followed by lower similarity with the third group and the lowest similarity with the fourth group.

Discussion

In the present study, 20 species of 11 genera of tetrigids were represented in an analysis using morphometric measurements. The results demonstrated some major differences in hind wing venation among the different species. Differences were observed in the length between the starting point of the costal vein and the tip of the fourth anal vein, between the tip of the costal vein and the tip of the third anal vein, and between the tip of the costal vein and the tip of the eleventh anal vein, and a contrast was observed between wing length and width. The different species could be clearly distinguished using the score of the three main components drawn using a scatter diagram. The results revealed that the morphological characteristics of hind wing venation of Tetrigidae can be used for the identification of species. Moreover, principal component analysis used to determine the phylogenetic relationships among the 20 species of tetrigids showed that these species cluster into four groups, with the first group having the highest similarity with the second group, followed by a lower similarity with the third group and the lowest similarity with the fourth group, i.e., *Coptotettix longjiangensis*.

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